

IN THE TITLE

Please amend the title as to read follows:

CHLAMYDIA PROTEIN, GENE SEQUENCE AND USES THEREOF

IN THE SPECIFICATION:

Please amend the specification as follows:

On page 1, line 3, insert this paragraph before, “1. FIELD OF THE INVENTION”:

This present application is a Continuation of Application Serial No. 08/942,596 filed on October 2, 1997, which is incorporated by reference in its entirety.

On page 17, lines 8-13, amend to read:

Figure 2. Consensus Nucleic Acid Sequence encoding the open reading frame of the HMW protein from *C. trachomatis* LGV L₂ (SEQ ID NO.: 1).

Figure 3. Deduced Amino Acid Sequence of the HMW protein from the PCR open reading frame from *C. trachomatis* LGV L₂ (SEQ ID NO.: 2).

On page 17, line 34, through page 18, line 8, amend to read:

Figure 6. Predicted amino acid sequences of HMW protein for *C. trachomatis* L₂, B, and F. The *C. trachomatis* L₂ sequence (SEQ ID NO.: 43) is given in the top line and begins with the first residue of the mature protein, E (see amino acid residues 29-1012 of SEQ ID NO.: 2). Potential eucaryotic N-glycosylation sequences are underlined. A hydrophobic helical region flanked by proline rich segments and of suitable length to span the lipid bilayer is underlined and enclosed in brackets. Amino acid differences identified in the B (see amino acid residues 29-1013 of SEQ ID NO.: 15) and F (see amino acid residues 29-1013 of SEQ ID NO.: 16) serovars are designated below the L₂ HMWP protein sequence.

On page 38, at lines 25-26, amend to read as follows:

<u>Plasmid</u>	<u>Microorganism ATCC Accession No.</u>	<u>Date Deposited</u>
<u>E.coli BL21 pAH342</u>	<u>ATCC 985538</u>	<u>September 8, 1997</u>
<u>E. coli TOP10 ATCC PTA-3719</u> <u>(pJJ 36-J)</u>		<u>September 20, 2001</u>

On page 43, lines 9-15, amend to read:

Using the N-terminal primary sequence as a guide, four degenerate oligonucleotide probes complementary to the nucleotides encoding the first six residues of the HMW peptide E-I-M-V-P-Q (SEQ ID NO.: 42 corresponding to residues 1-6 of SEQ ID NO.: 3) (residues 1-6 of SEQ ID NO.: 3), and comprising all possible nucleotide combinations (total degeneracy = 192 individual sequences), have been designed and employed as forward amplification primers.

On page 47, lines 7-19, amend to read:

DNA sequence data produced from individual reactions were collected and the relative fluorescent peak intensities analyzed automatically on a PowerMAC computer using ABI Sequence Analysis Software (Perkin-Elmer). Individually autoanalyzed DNA sequences were edited manually for accuracy before being merged into a consensus sequence "string" using AutoAssembler software (Perkin-Elmer). Both strands of the HMW protein gene segment encoded by pAH306 were sequenced and these data compiled to create a composite sequence for the HMW protein gene segment. The sequence encoding the segment of HMW protein is listed as SEQ ID NO.: 10 and is represented by nucleotides 382466 to 19791976 in Figure 2. A map of pAH306 is shown in Figure 5.

On page 50, lines 3-15, amend to read:

Plasmid pAH316 is one derivative isolated by these procedures. Restriction analysis of pAH316 demonstrated that this derivative contains a *C. trachomatis* L₂ insert of ~4.5Kbp which consists of two EcoRI fragments of ~2.5Kbp and ~2.0Kbp in size. Southern hybridization analysis using the ~0.2Kbp E/H fragment as a probe localized this sequence to the ~2.5Kbp EcoRI fragment of pAH316. Directional PCR analyses employing purified pAH316 plasmid DNA as a template and amplification primer sets specific for ~0.2Kbp E/H fragment and T3 and T7 vector sequences demonstrated pAH316 encodes the C-terminal segment of the HMW protein gene. The coding segment of the HMW protein is represented by nucleotides 19741977 to 3420 in Figure 2, and is listed as SEQ ID NO.: 11.

On page 51, line 29 through page 52, line 7, amend to read:

Mini-prep DNA from ampicillin-resistant transformants picked at random were prepared, digested to completion with XhoI, EcoRI, or a combination of both and examined for the presence and orientation of the ~1.5 Kbp truncated HMW protein ORF insert by agarose gel electrophoresis. Mini-prep DNA from clones determined to carry the ~1.5Kbp XhoI/EcoRI insert was prepared and used to program asymmetric PCR DNA sequencing reactions to confirm the fidelity of the junction formed between the HMW protein fragment and the (His)₆ affinity purification domain of the expression vector. Plasmid pJJ36-J was one recombinant derivative isolated by these procedures and is represented by nucleotides 446466 to 19771980 in figure 2. The deduced amino acid sequence of the truncated fragment of HMW protein is represented by amino acids 29 to 532533 in Figure 3 and is listed as SEQ ID NO.: 17.

On page 56, lines 4-15, amend to read:

DNA sequence data were collected using the ABI 310 Sequenator and analyzed automatically on a PowerMAC computer and appropriate computer software as described in Example 4. Individually autoanalyzed DNA sequences were edited manually for accuracy before being merged into a consensus sequence “string” using AutoAssembler software (Perkin-Elmer). Both strands of the HMW protein gene from the *C. trachomatis* B and F serovars were sequenced and these data compiled to create composite consensus sequences for both the *C. trachomatis* B and F HMW protein genes. These sequences The amino acid sequences encoded are listed as SEQ ID NOS.: 14 and 15 and 16. Sequence comparisons of the L₂, F and B strains are presented in Figure 6.

On page 58, lines 6-18, amend to read:

Mini-prep DNA from ampicillin-resistant transformants picked at random were prepared, digested to completion with KniI, HindIII, or a combination of both and examined for the presence and orientation of the ~3.2 Kbp HMW protein ORF insert by agarose gel electrophoresis and ethidium bromide staining. Mini-prep DNA was used to program asymmetric PCR DNA sequencing reactions as described in example(s) above to confirm the fidelity of the junction formed between the HMW protein fragment and the (His)₆ affinity purification domain of the vector. Plasmid pAH342 was one derivative isolated by these procedures, which contains the HMW protein gene ORF from *C. trachomatis* L₂ and is represented by nucleotides 446466 to 3421 in Figure 2.

On page 60, lines 20-35, amend to read:

Samples were loaded onto Tris/glycine preparative acrylamide gels (4% stacking gel, 12% resolving gel, 30:0.8 acrylamide:bis solution, 3mm thickness). A prestained molecular weight standard (SeeBlue, Novex) was run in parallel with the rHMW protein samples to

identify size fractions on the gel. The area of the gel containing proteins having molecular masses of ~~-50-70~~ ~110 Kdal was excised and the proteins electroeluted using an Elu-Trap device and membranes (S&S) as specified by the manufacturer. Electroeluted protein was dialyzed to remove SDS. The protein concentration of the sample was determining using a Micro-BCA system (Pierce) and BSA as a concentration standard. The purity of rHMW protein was determined using conventional SDS-PAGE and commercially available silver staining reagents (Silver Stain Plus, Novex) as shown in Figure 4.